By 'dissociating moiety' in claim 13 and other independent claims, the Applicants mean the following. A portion of the target molecule can be chemically converted to other molecular species, i.e., dissociating moieties, while residing in the hydrophobic lipid environment of the liposomal membrane. A dissociating moiety may be observed after the incorporation of the target molecule, i.e., the polynuclear complex, with the lipid membrane constituents. Alternatively, the dissociating moiety also may be observed following the formation of the liposomal carrier construct. These molecular rearrangements can occur over a time period ranging from just a few minutes to several days and over a temperature range from 4°C to 60°C. These other molecular species or dissociated moieties and combinations thereof can form as a result of chemical exchange reactions that take place in the liposomal membrane that houses the targeted polynuclear complex. This chemical conversion is likely to alter the structure of at least some of the targeting molecules residing *in situ* which are routinely characterized and represented by the structures of the polynuclear complex notably defined as combinations of the octahedral structural unit of Cr-bis[N-(2,6-diisopropylphenylcarbamoylmethyl) iminodiacetic acid] as shown in Figure #4. For example, a new target complex can form as a result of several exchange reactions within the liposomal membrane which include but are not limited to the formation of 1,2-distearoyl sn-glycerol-3-phosphocholine-Cr-[N-(2,6-diisopropylphenylcarbamoylmethyl) iminodiacetic acid], dicetyl phosphate-Cr-[N-(2,6-diisopropylphenylcarbamoylmethyl) iminodiacetic acid] and appropriate salts thereof.

By 'first member being soluble in second member comprising a liposome' in claim 18, the Applicants mean the following. The second member is a liposome that contains hydrophobic molecules in the lipid bilayer. These molecules are represented by cholesterol, dicetyl phosphate and distearoyl lecithin. These hydrophobic molecules are soluble in organic solvents, i.e.,

chloroform:methanol (2:1, v/v). The target molecule is designated as the first member and it too is soluble in organic solvents, i.e., chloroform:methanol (2:1, v/v). Therefore, since, as a general rule, like substances dissolve like substances, the first member will mix and be soluble in the second member and vice versa. Note that both the first member, i.e., the target molecule and the second member, i.e., the liposomal membrane, are both soluble in organic solvents and also soluble in each other.

By 'soluble in liposome' as recited in claim 22, Applicants mean the following. It is assumed that the Examiner's supposition here is that a liposome is a bilayer structure and not a specific compound and that this bilayer exists as a separate phase in water. The bridging agent complex otherwise known as the target molecule is insoluble in water but notably soluble in organic solvents, such as chloroform:methanol (2:1, v/v). The bridging agent complex can be mixed with and become soluble in the array of hydrocarbon-based lipid molecules that comprise the liposomal membrane. All of the lipid molecules as well as the target molecule that have been mentioned exhibit affinity for one another and are soluble in one another. The Examiner's supposition is correct in that the liposome is a bilayer structure and that this bilayer exists as a separate phase in water. The Examiner is also correct in noting that it is not a specific compound. However, the liposome does exhibit a specific biochemical membrane structure that is composed of the various lipid components which allows other compounds, such as the polynuclear target complex, with similar molecular properties and like solubilities to be soluble in the lipid moieties that comprise the aforementioned membrane.

By 'target molecule' as recited in claim 26, the Applicants mean the following. Chromium is not the target molecule per se. It is simply a bridging ion that is used to connect two identical molecules of N-(2,6-diisopropylphenylcarbamoylmethyl) iminodiacetic acid in

order to form the basic building block which is the octahedral structure depicted in Figure #3. It is important to note that this building block eventually combines with other octahedral building blocks using hydroxyl ions as connecting moieties to form the polynuclear complex of Figure #4 which is the target molecule.

By 'polynuclear complex' as recited in claim 32, the Applicants mean the following. The polynuclear complex of this invention is signified by the structures shown in Figure #4 which shows various configurations of the chromium target molecule complex. These structures are confirmed by the data generated from mass spectroscopy analysis of the chromium target complex as shown in Figure #6. Figure #4 diagrammatically illustrates that various combinations of the octahedral complex can exist. In Figure #4 one octahedral complex can be chemically bound to another octahedral complex and so on. It is this joining of octahedral complexes with one another mediated by the hydroxyl connecting ions that change the chemical properties of the chromium target complex or the polynuclear complex such that the complex is insoluble in water. This new property allows the polynuclear complex to become soluble and mix freely with the liposomal lipid constituents which form the bipolar liposomal membrane.

The Examiner argues (rejection #5) that "claims 5, 7-28, 30 and 32-36 are rejected under 35 U.S.C. 102(b) as being anticipated by WO 88/00474 of record or Geho (4,603,044) also of record. The Applicants would like to respond as follows. The cited inventions are distinguished from the present invention because they use a sequential addition of selected molecular components to form the target molecule that builds onto the surface of a liposomal membrane where water solubility is predominant. Water solubility of the constituents of the cited inventions plays an important chemical role by promoting the ensuing cascade of chemical reactions that add to the surface of a liposome in a linear, ordered and designated manner. The

present application, however, uses a water insoluble polynuclear complex that is added in a single gravimetric step to the other lipid constituents in the beginning of the liposomal synthesis. At this juncture, the components of the lipid crust are weighed out along with the target molecule complex, dissolved in an appropriate organic solvent, mixed and then dried. Following the addition of an aqueous phase and sonication energy input, the target molecule then associates and becomes soluble in the hydrophobic environment provided by the other membrane constituents. Without the creation of the water insoluble polynuclear complex the invention in terms of synthesis would be non-scalable. The formation of the polynuclear complex target molecule allows the invention to work in a simple and verifiable fashion.

The essential difference between the present invention which is the subject of this pending application and the patents of either Bosworth or Baldeschwieler or, for that matter, any other patents in this general area of inquiry is that the present invention is directly related to the discovery of a new composition of matter that defines the liposomal target molecule. The target molecule that is unique to the present invention is a chemically complex molecular entity that directs the liposomal structure to the hepatocytes in the liver of a warm-blooded host. The target molecule is formulated using novel chemical reaction conditions that require conducting a highly specific step-by-step methodology. This procedure results in the production of a novel chemical composition that is defined as a polynuclear complex. This complex has been found to be useful for targeting individual liposomes to cellular receptors of the hepatobiliary system in man.

The present invention describes a synthetic procedure that is encompassed within a general scientific methodology outlined under the Detailed Description of the Invention in the specification. This procedure results in the creation of a new hepatocyte-targeting complex that demonstrates a new and unique composition of matter. The structures of this target molecule are

shown diagrammatically in the attached **Appendix A**. Figures #3 and #4 in the attached **Appendix A** represent the formation of a targeted polynuclear complex which is composed of trivalent chromium ions, structured water, hydroxyl bridging ions and an organic moiety represented by 2,6-diisopropylphenylcarbamoylmethyl iminodiacetic acid. All of these molecular moieties are bound together and exhibit an octahedral structural configuration. The octahedral structure contains chromium ions connected to carboxyl functionalities and imino nitrogen groups and represents the repeating structural unit in the polynuclear complex as shown in Figure #3. The coordination of these molecular entities requires a specific set of reaction conditions and produces targeted polynuclear complexes of very high molecular weight. For example, complexes with mass numbers of 1,948 and 1,549 are routinely observed in target molecules following an analytical determination that employs the methods of mass spectrometry as shown in Figure #5. The molecular weight of the analytical sample representing the polynuclear complex is continuously degraded by electron bombardment resulting from a high-energy electron beam produced by the mass spectrometer. It is therefore reasonable to assume that higher molecular weight multiples of the targeting polynuclear complex are likely to be found in the sample prior to an analytical determination by mass spectrometry.

The newly analyzed compounds with mass numbers of 1,948 and 1,549 as shown in Figures #4 and #5 represent polynuclear multiples of the molecular entities that include chromium, water, hydroxyl ions and the 2,6-diisopropyl organic moiety. The properties created during the formation of the coordinated polynuclear complexes are chemically and morphologically different from any previously observed compounds and are new to the art.

For example, the polynuclear complexes of this invention are <u>insoluble</u> in aqueous media and therefore are unable to occupy the core volume of a liposomal delivery system. However, by

contrast, these same complexes are soluble in organic solvents, such as chloroform:methanol (2:1, v/v) and acetonitrile. As a result, they seek to occupy hydrophobic environments and thus are able to be used along with the other lipid membrane constituents. Later, following energy input, these complexes become associated within the liposomal bilayer structure.

The conditions necessary for the formation of the targeted polynuclear complexes of this present invention are unique and found to be markedly different from any other set of conditions or methodologies that has been previously reviewed in a screening of related patent and pertinent subject matter. These conditions also differ from any conditions or methodologies that have been found in the general literature in regard to the formation of targeting compounds. The formation of the polynuclear complex of this invention occurs at an acid pH between pH 3.2 and pH 3.3 over a time period of several days. However, this reaction can only occur after first manipulating the pH of the stock solutions of A and B within a specified and narrow pH range in an acid environment. This particular set of conditions results in the formation of the polynuclear complex target molecule that is described morphologically as a dark-green colored orthorhombic compound expressing a quasi-crystalline needle-like structure that ranges in size from 0.1 to 3.0 mm in length. However, since this compound lacks a repeatable and definable crystalline unit cell, it is technically considered to be amorphous as determined by x-ray crystallography. The polynuclear complex is insoluble in water, buffer or aqueous media and has hepatocyte targeting capabilities when incorporated in a liposomal membrane. The solubility properties of the complex allow it to function as an essential liposomal membrane component when it is mixed with the other liposomal ingredients. This procedure is achieved by employing a single manufacturing step that requires only a simple gravimetric addition of the targeting complex to the other membrane constituents.

Previously disclosed hepatocyte targeting technologies introduced by the Applicants have never formally addressed the critical and important formulation difficulties and manufacturing hurdles that would eventually have to be overcome in order to facilitate very large scale production of the targeted liposomal product. Instead, these earlier technologies were made workable primarily within an experimental setting that had a restricted set of operating conditions. These previous formulation procedures limited production because of multi-step methodologies.

The formation of this water-insoluble targeted polynuclear complex has never been observed to occur at physiological pH. However, in contrast, these same physiological conditions are prerequisite for all nuclear magnetic resonance (NMR) imaging and various scintillation technologies that produce, for example, a NMR scan or a radioimage. These aforementioned technologies are singly useful for diagnostic purposes and are not by definition used for therapeutic intervention.

This present invention overcomes these previous limitations as evidenced by the manufacturing simplicity of the final therapeutic liposomal drug product as described in the specification under the Detailed Description of the Invention, thereby providing a therapeutic benefit for the end user of this targeted liposomal drug carrier construct.

The applicants further wish to address specific differences between this present invention and cited inventions by Bosworth and Baldeschwieler.

The Bosworth patent teaches the creation of a non-toxic paramagnetic image-altering agent containing a chelate of a paramagnetic element. The paramagnetic chelate is carried by a liposome. The invention is directed to a method for enhancing NMR imaging of body organs and selected tissues using non-targeted liposomes.

It is important to note that the Bosworth invention describes the use of water-soluble chelates, and, unlike the present invention, does not describe the use of insoluble chelates. Moreover, unlike the present invention, the Bosworth invention does not teach the creation of a liposome with an attached hepatocyte-targeting molecule; rather, the Bosworth liposomes are not targeted to hepatocytes. Instead, they are made to accumulate in the reticuloendothelial system (RES) cells of the liver, such as macrophages. In contrast, the purpose of the present invention is to provide an enhanced method of targeting a variety of active agents (metabolic agents) specifically to metabolic cells of the liver known as hepatocytes. Finally, while the Bosworth invention emphasizes the paramagnetic properties of the metal for the purpose of non-hepatocyte specific NMR imaging of the liver, the present invention instead utilizes the chemical properties associated with the bonding chemistry of the chromium ion for the purpose of creating a stable hepatocyte-specific targeting moiety that is attached to the liposome.

Other differences between the present invention and the Bosworth invention include critical methods of manufacture. Bosworth filters his liposomes through a 10,000 Angstrom membrane filter. This allows for the selection of large liposomes, which preferentially find their way to the RES cells (ie, macrophages) in the liver. In contrast, the present invention describes a filtration method that preferentially selects very small liposomes that, when targeted with the hepatocyte-specific targeting moiety, preferentially accumulate in a different liver cell type, the hepatocytes. In addition, the Bosworth invention uses low transition temperature lipids. The present invention uses high transition temperature lipids.

Likewise, the cited Baldeschwieler invention (U.S. 4,310,506) is significantly different from the present invention. Like Bosworth, and unlike the present invention, Baldeschwieler is concerned with the use of liposomes to carry chelating agents to the liver for imaging purposes.

The Baldeschwieler invention describes the use of ionophores to enhance loading efficiency of chelators useful as imaging agents, into liposomes. The present invention, however, does not address liposome loading. Rather, the purpose of the present invention is to target liposomes to hepatocytes. The present invention makes no use of ionophores.

Although the Baldeschwieler invention and the present invention describe the use of chromium, there is a critical difference in the use of chromium: the Baldeschwieler invention uses chromium as a radioactive tracer for imaging purposes. The present invention instead uses chromium to stabilize and position the hepatocyte-targeting moiety, which is in turn attached to the liposome. Moreover, like the Bosworth invention, a water-soluble chelate is described; however, the present invention describes a water-insoluble chelate.

It is presumed that the imaging liposomes described by Baldeschwieler, not having an hepatocyte targeting moiety, end up in liver macrophage cells, a result that is avoided by the present invention.

In summary, neither the Bosworth nor the Baldeschwieler patents describe a water insoluble metal targeting moiety complexed with a liposome for the purpose of hepatocyte-specific delivery of therapeutic (eg, metabolic) agents. Rather, they describe the use of water-soluble metals in conjunction with non-targeted liposomes, designed for accumulation in RES cells in the liver and other organs for the purpose of imaging.

The Applicants hereby request a meeting with the Examiner to further discuss the Examiner's questions.

Respectfully submitted,

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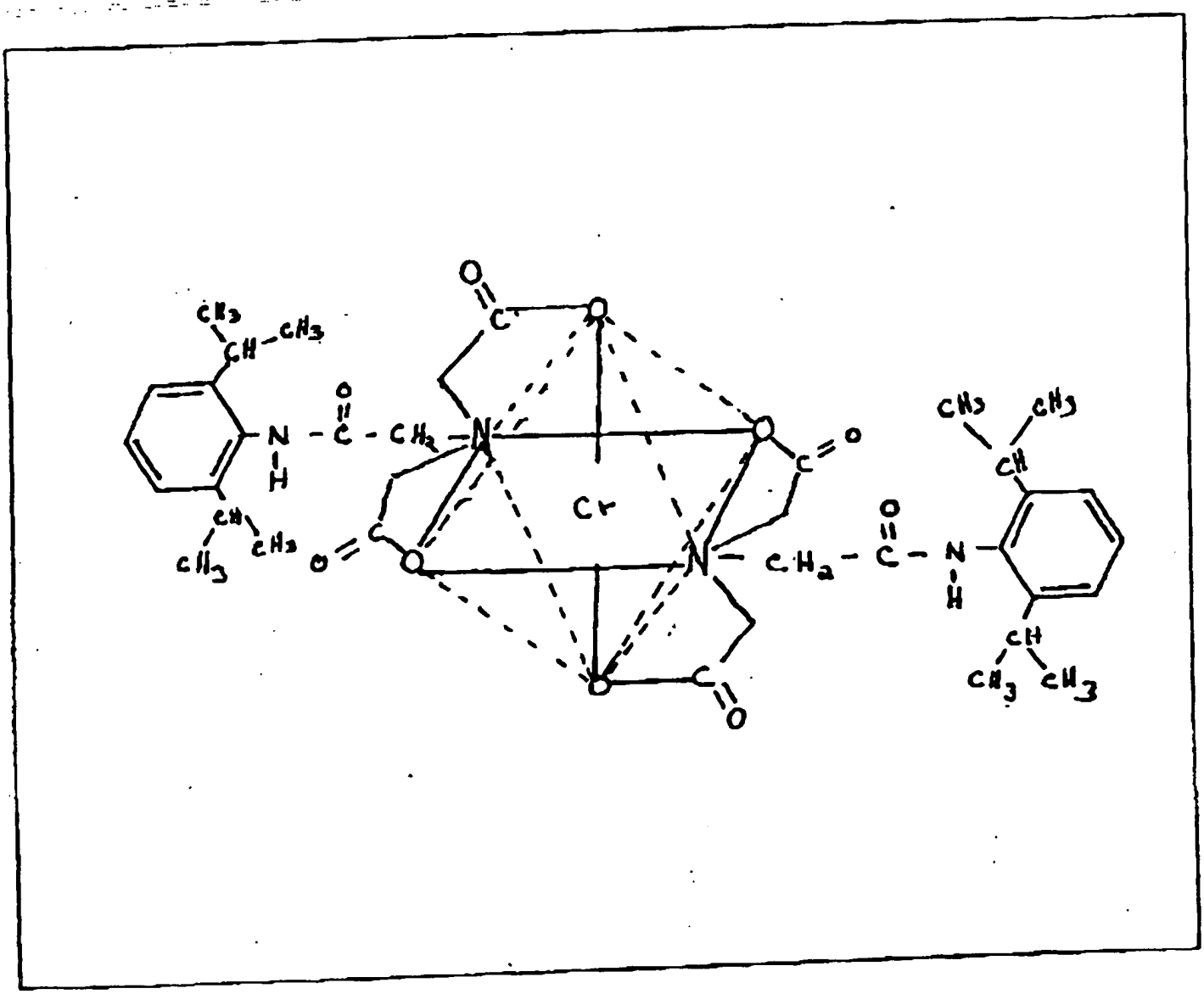
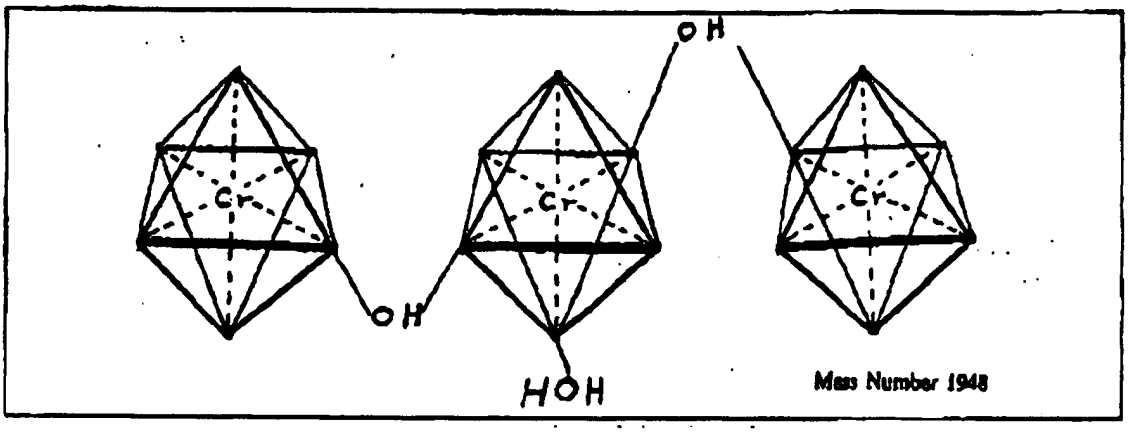
Appendix A

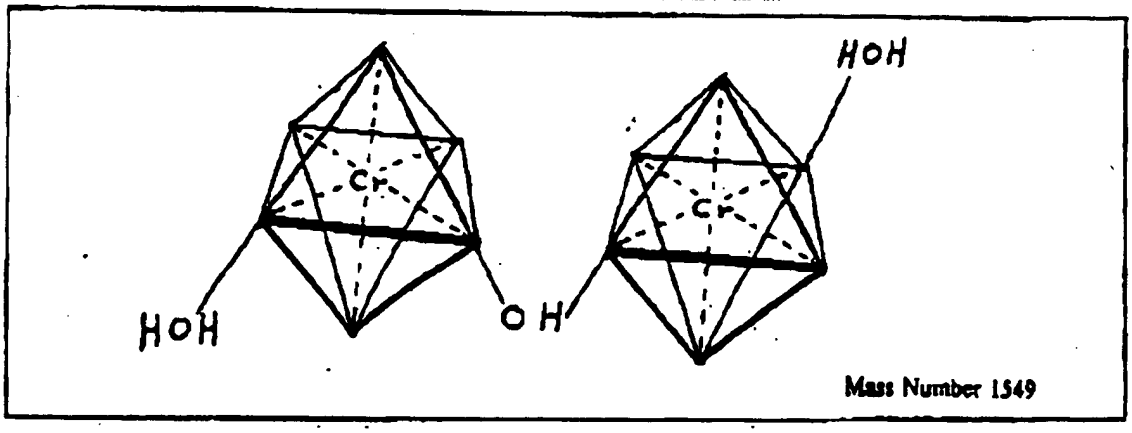
Figure 3



Appendix A

Figure 4





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Appendix A

Figure 5